APPENDIX

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Specific Interaction of $TAF_{II}105$ with OCA-B Is Involved in Activation of Octamer-dependent Transcription*

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$TAF_{II}105$ is a TFIID-associated factor highly expressed in B lymphocytes. This subunit is found in a small portion of TFIID complexes and is homologous to human $TAF_{II}130$ and *Drosophila* $TAF_{II}110$. In the present study we show that $TAF_{II}105$ is involved in transcription activation directed by the B cell-specific octamer element found in many B cell-specific genes. B cells overexpressing $TAF_{II}105$ display higher octamer-dependent transcription, whereas expression of a C-terminal truncated form of $TAF_{II}105$ inhibits octamer transcription in a dominant negative manner. In addition, antibodies directed against $TAF_{II}105$ specifically inhibit octamer-dependent transcription. Reporter gene analysis revealed that $TAF_{II}105$ elevates octamer transcription in the presence of OCA-B, a cofactor subunit of Oct1 and Oct2 proteins. *In vitro* binding assays and functional studies established that the effect of $TAF_{II}105$ on octamer activity involves interaction of $TAF_{II}105$ with octamer-binding complexes via the C-terminal activation domain of OCA-B. These findings link $TAF_{II}105$ coactivator function to B cell-specific transcription.

The program of B cell-specific gene expression during development is highly controlled and is governed by gene-specific organization of DNA regulatory elements and combinatorial interactions of ubiquitous and cell type-specific transcription factors and cofactors. In recent years significant progress has been made in identifying and understanding the physiological role of transcription factors involved in B cell development (1). However, much less is known about the mechanism underlying the transcription activation process by these factors. Considering the accumulating evidence indicating that interactions of activators with various components of the general transcription apparatus are essential for transcription activation process, it is reasonable that similar mechanisms apply also for B cell transcription factors.

Several studies implicated the basal transcription factor TFIID as potential target for specific activation domains of activators (2, 3). TFIID is a multiprotein complex consisting of TATA-binding protein (TBP) and associated factors, TAFs[1] (4–8). TFIID is required for directing core promoter recognition and pre-initiation complex assembly (9, 10). *In vitro* transcription studies have indicated that the TAF subunits play a crucial role as activation domain mediators (coactivators) through direct interaction with activators (11–13). In addition, certain TAFs also function as promoter selectivity factors (14, 15). Several reports suggested that certain TAFs play an important role in gene-specific transcriptional activation *in vivo* in high eukaryotes (16–20). Recent genetic experiments in yeast with individual TAF mutants suggested that, whereas some TAFs are essential for transcription of the majority of class II genes (21–26), certain TAFs are not generally required for transcription activation (27–29). Due to a lack of additional genetic studies in metazoans, little is known about the transcription regulatory pathways that specifically require the activities of the TAFs.

Previously we have cloned a subunit of TFIID, $TAF_{II}105$, that is related to the coactivator subunit $hTAF_{II}130$ and to *Drosophila* $TAF_{II}110$ (30). The C-terminal third of these subunits is highly conserved (17, 30). The N terminus of $TAF_{II}105$ is significantly more diverged and was shown to be involved in interaction with activation domains of activators (19). Unlike most TAF subunits that are conserved from yeast to human, no homolog has been found for the family of $hTAF_{II}105$, $hTAF_{II}130$, and $dTAF_{II}110$ proteins in yeast, suggesting that these TAFs might be involved in transcription regulatory pathways that do not exist in yeast.

$TAF_{II}105$ was originally identified as TFIID-associated polypeptide that is highly abundant in purified TFIID complex isolated from mature B cell line (30). Because the expression pattern of a transcription factor usually correlates with its function, it was postulated that $TAF_{II}105$ may be involved in B cell-specific transcription (30). $TAF_{II}105$ appears to be present only in a subset of TFIID complexes, and therefore is likely to function in the context of a specific set of genes. Consistent with these assumptions, our previous analysis of $TAF_{II}105$ function revealed that it acts as an activation domain-specific coactivator of p65/RelA, a subunit of the NF-κB family of transcription factors (19). These studies also indicated that the p65·$TAF_{II}105$ complex is involved in cytokine-mediated gene expression and anti-apoptotic gene activation (19).

In the present study we investigated the role of $TAF_{II}105$ in transcription regulation mediated by the octamer motif. The octamer motif plays a crucial role in directing the B cell-specific expression of immunoglobulin and other B cell-specific genes. This motif is found in the promoters and enhancers of all immunoglobulin genes and is essential for the B cell-specific expression of these genes (31). Two octamer-binding factors are expressed in B cells, a ubiquitous protein Oct1 and the B cell-specific factor Oct2. These proteins associate with a B cell-specific cofactor OCA-B also known as OBF-1 and Bob1 (32–34). OCA-B interacts with Oct1 and Oct2 via its N terminus and provides a strong activation domain to this complex.

Our studies reveal that $TAF_{II}105$ plays a transcription coactivator role required for a high level of B cell-specific octamer

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[1] The abbreviations used are: TAF, TATA-binding protein associated factor; MLP, major late promoter; PCR, polymerase chain reaction; GST, glutathione *S*-transferase; HA, hemagglutinin.

activity. Dissection of the molecular mechanism involved in the effect of $TAF_{II}105$ on octamer transcription revealed that $TAF_{II}105$ interacts with octamer-binding complexes through direct interaction with the cofactor OCA-B. This interaction is required for $TAF_{II}105$ enhancement of octamer-dependent activity and is mediated by the C-terminal activation domain of OCA-B and the coactivation domain of $TAF_{II}105$. These results suggest that $TAF_{II}105$ is an additional regulatory component of octamer-dependent B cell-specific gene expression, acting as coactivator for octamer-binding complexes.

MATERIALS AND METHODS

In Vitro Transcription

For the *in vitro* transcription reactions we used nuclear extract prepared from the B cell line Daudi. The reaction mixture (18 μl) contained 3 μl of extract, 6 μl of transcription buffer (20 mM Hepes, pH 7.9; 100 mM KCl; 2 mM $MgCl_2$; 0.2 mM EDTA; 20% glycerol; and 1 mM dithiothreitol), 0.6 μl of 0.1 M $MgCl_2$, 40 units of RNasin, and 200 ng of DNA template. The reaction was incubated at 30 °C for 30 min. Then 2 μl of the nucleotide mixture was added (for IgH and 2xOct/IgH G-less reporters: 5 mM ATP and UTP, 1 mM 3′-*O*-methyl-GTP, and 100 μM [α-^{32}P]CTP; and for the major late promoter (MLP) U-less reporter: 5 mM ATP and GTP and 100 μM [α-^{32}P]CTP), and the reaction was incubated for additional 10 min at 30 °C. The reaction was stopped by adding 200 μl of stop mixture (2 M NH_4AC; 10 mM EDTA; 10 mM Tris, pH 7.4; and 0.25 μg/ml tRNA), phenol chloroform extracted, and ethanol precipitated and run on a 6% sequencing gel. When antibodies were used in the assay, they were pre-incubated with the transcription mixture in the absence of the DNA template for 30 min, followed by the procedure described above. Anti-$TAF_{II}105$ antibodies used in the transcription are polyclonal antibodies directed against amino acids 1–552 (30). The anti-HBV core antibodies were kindly provided by Dr. Y Shaul.

Plasmids

Expression Vectors—Full-length OCA-B was generated by polymerase chain reaction (PCR) using a Daudi cell cDNA library and the following oligonucleotides: 5′-CCGAATTCCATATGCTCTGGCAAAAA-CCCACAG -3′; 5′- TCCCAGTTTCAGGGAACAGGA-3′. For *in vitro* transcription and translation of OCA-B, the PCR fragment was digested by *Nde*I and was cloned into *Nde*I-*Stu*I sites of a pTβstop vector. The sequence of the PCR fragment was determined and found to be correct. A mammalian expression vector for OCA-B (pCGN-OCA-B) was generated in two steps. An *Nde*I-*Ecl*136II fragment from pTβ-OCA-B was first subcloned into *Nde*I-*Hin*cII sites of pBSNLS-HA downstream to a nuclear localization signal and HA tag. Next, a *Sma*I fragment from this plasmid was inserted into the *Sma*I site of pCGN vector (35). $TAF_{II}105$ expression plasmid was created by inserting an *Nco*I (filled in) and a *Bgl*II fragment from pVLHA-$TAF_{II}105$ into *Sma*I-*Bam*HI sites of pCGN. $TAF_{II}105$ retroviral expression vector (pBabe-105) was generated using a three-point ligation reaction. An *Ecl*136II-*Nco*I fragment from pCGN-105ΔC containing the thymidine kinase leader sequence and an *Nco*I-*Sal*I fragment from the pTβ-$TAF_{II}105$ plasmid, encoding for $TAF_{II}105$, were ligated into *Sna*BI-*Sal*I sites of a pBabe-neo vector. pTβ-$TAF_{II}105$ was created by inserting an *Nde*I fragment from pV-LFlag-$TAF_{II}105$, previously described (30) into the *Nde*I site of pTβstop. $TAF_{II}105\Delta N$ plasmid fused to glutathione *S*-transferase (GST) was generated by PCR using: 5′-CGATGAGGATGACATCAATG-3′ and T7 primers. Bluescript-$TAF_{II}105$ was used as a template with the proofreading PWO enzyme (Roche Molecular Biochemicals). The PCR product was ligated into *Stu*I-*Eco*RI sites of the pTβstop vector, followed by subcloning into *Nco*I-*Eco*RI sites of vector pGEX.

$TAF_{II}130\Delta C$ (a), fused to GST, was cloned by digesting the $TAF_{II}130\Delta C$ plasmid with *Nco*I-*Hin*cII, at amino acids 484–757, and cloning into *Nco*I-*Stu*I of vector pGEX-2TKN. The fragment GST-130ΔC (b), amino acids 100–468, was cloned using a *Nco*I-*Stu*I digest and inserted into the pGEX-2TKN vector in the *Nco*I-*Stu*I sites.

Reporter Constructs—The Oct-TATA reporter plasmid was constructed by inserting a double-stranded synthetic oligonucleotides (see below) containing a single octamer motif as a *Hin*dIII-Blunt fragment into the *Hin*dIII-*Ecl*136II sites upstream to a minimal α-actin core promoter (−40 to +80) of pLuc-α-actin vector. Octamer oligonucleotides were as follows: 5′-AGCTTTCAGGGTATGCAAATTATTAAGTCTCG-3′ and 5′-CGAGACTTAATAATTTGCATACCCTGAA-3′.

The construct Oct TATA/SV40 was generated by subcloning an Oct TATA *Hin*dIII-*Nar*I fragment from pOct-TATA into *Hin*dIII-*Nar*I of pGL2-enhancer vector (Promega). An Oct-less/SV40 construct was created by inserting a *Sac*I-*Nar*I fragment from pLuc-α-actin plasmid into the *Sac*I-*Nar*I sites of pGL2-enhancer plasmid. The 4xOct wild type and 4xOct mutant plasmids were a generous gift from Dr. T. Wirth. The J chain reporter plasmid was kindly provided by the late M. Koshland, and the Igκ luciferase was described previously (30). The reporter plasmid 2xOct/IgH, used for *in vitro* transcription, was constructed by using two oligonucleotides; 5′-AATAATTTGCATACCCTAATTTGCAT-ACG-3′ and 5′-AATTCGTATGCAAATTAGGGTATGCAAATTATT-3′. The two oligonucleotides were annealed and ligated into IgH (G-less cassette) in *Eco*RI-*Ecl*136II sites.

OCA-B Mutants—To obtain the OCA-B 1–230 mutant, pCGN-OCA-B was first digested with *Xba*I, followed by a partial digest with *Hin*cII, and the fragment was subcloned into *Xba*I-*Sma*I of the pCGN vector. pTβ-OCA-B 1–230 was constructed by inserting an *Nde*I-*Hin*cII (partial digest) fragment into *Nde*I-*Stu*I sites of pTβstop vector.

Details on the generation of Gal4-OCA-B fusion proteins can be given by request.

In Vitro Binding Experiments

The N and C termini of $TAF_{II}105$, and $TAF_{II}130$ N-terminal fragments were expressed as a GST fusion protein in *Escherichia coli*. The recombinant proteins were purified and immobilized on glutathione-Sepharose beads. ^{35}S-labeled Oct1, Oct2, and various OCA-B-derived proteins were synthesized *in vitro* using T7 RNA polymerase and rabbit reticulocytes lysate (Promega TNT kit), and incubated with the different GST-purified proteins in 0.1 M KCl HEMG buffer (20 mM Hepes, pH 7.9; 100 mM KCl; 12.5 mM $MgCl_2$; 0.2 mM EDTA; 0.1% Nonidet P-40; 1 mM dithiothreitol; 0.2 mM phenylmethylsulfonyl fluoride) for 2 h at 4 °C. The beads were washed three times with the same buffer and twice with 0.2 M KCl HEMG buffer. The bound proteins were eluted by boiling for 5 min in protein sample buffer followed by SDS-polyacrylamide gel electrophoresis (PAGE) and autoradiography.

Nuclear extract from BJAB cells was prepared as described (30). 50 μl of nuclear extract (15 mg/ml) in 0.1 M HEMG + 0.1% Nonidet P-40 were incubated with GST105ΔC or with GST beads as described above. After the washes, bound proteins were eluted with 1 M NaCl HEMG, loaded onto an SDS-PAGE, and analyzed by Western blot, using anti-OCA-B, anti-Oct2, and anti-RelB antibodies (Santa Cruz Biotechnologies).

Transfections and Cell Lines

293 cells (embryonic kidney fibroblasts) were maintained in F-12 Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum. Transfections were performed using the standard $CaPO_4$ method. For reporter assays, subconfluent cells were transfected with the indicated plasmids in a 24-well multidish using a total of 0.8 μg of plasmid, usually 100 ng of the reporter gene, 400 ng of pCGN-OCA-B, and 200 ng of pCGN-$TAF_{II}105$. The amount of CMV vector in each transfection was kept constant.

BJAB (human), S194 (mouse), mature B cells were maintained in RPMI medium supplemented with 10% fetal calf serum. Transfections to S194 stably expressing $TAF_{II}105$ were done according to the DEAE-dextran method, using 0.5–1 μg of reporter plasmid in a total of 8.5 μg of DNA, in 100-mm dishes. To analyze the effect of dominant negative $TAF_{II}105$, a transfection assay was performed using 3 μg of reporter plasmid and 12 μg of $TAF_{II}105\Delta C$ plasmid. The amount of CMV vector in each transfection was kept constant. A pool of S194 cells expressing $TAF_{II}105$ was generated in two steps. First, $TAF_{II}105$ retroviral particles were produced in 293 cells. Briefly, 293 cells were seeded at a density of 1×10^6 cells in 50-mm plates 24 h before transfection. Shortly before transfection, medium was replaced with fresh Dulbecco's modified Eagle's medium/10% fetal calf serum containing 25 μM chloroquine. 10 μg of DNA containing pBabe-$TAF_{II}105$ and ψ helper vector at a 1:1 ratio were transfected into the cells using the calcium phosphate-DNA coprecipitation method. After 8 h, the medium was replaced with a fresh one. Supernatant containing viral particles was harvested 48 h after transfection and filtered through a 0.45-μm filter. To infect S194 cells with the virus, 2 ml of the viral supernatant and polybrene (final concentration, 5 μg/ml) were used to infect 0.5×10^6 cells. After incubation at 37 °C for 2.5 h, an additional 8 ml of RPMI/10% fetal calf serum was added to each plate. Cells were incubated at 37 °C for an additional 1–1.5 days before addition of G418 (1 mg/ml; the active component is ~0.5 mg/ml). Medium was changed every

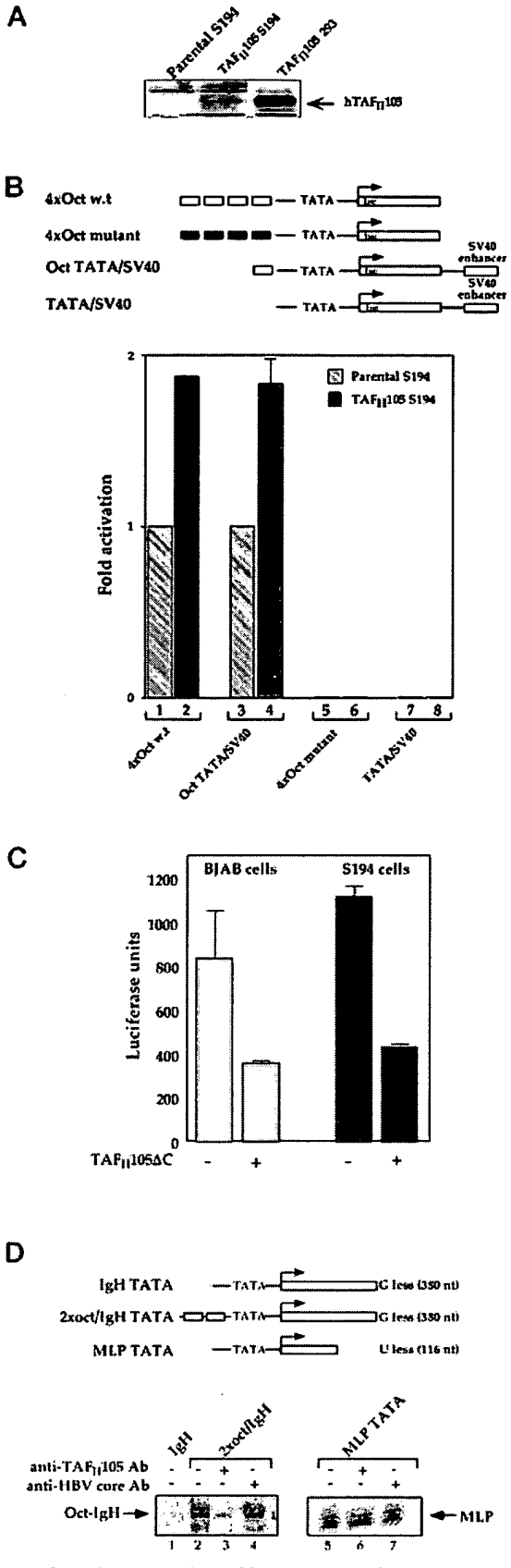

FIG. 1. **Ectopic expression of hTAF$_{II}$105 in S194 cells elevates octamer-dependent transcription.** *A*, stable S194 cells (mouse B cells) expressing human TAF$_{II}$105 were generated (as described under "Materials and Methods") and analyzed by Western blot using anti-hTAF$_{II}$105 antibodies. 293 cells overexpressing TAF$_{II}$105 were used as positive control. *B*, reporter plasmids (shown schematically at *top*) driven by four tandems repeats of the wild type octamer motif (*4xOct w.t*), a mutated version of the four octamers (*4xOct mutant*), a single octamer motif upstream to a minimal α-actin promoter (*Oct TATA/SV40*) and α-actin promoter alone (*TATA/SV40*) were transfected into parental S194 cells or into a pool of TAF$_{II}$105 expressing S194 cells as indicated. Luciferase activity was measured, and the activity of the wild-type reporters was normalized to 1. Standard error bars were calculated from at least three independent experiments. *C*, dominant negative TAF$_{II}$105 inhibits octamer-dependent transcription in B cells. BJAB and S194 B cell lines were transfected with oct-TATA/SV40 reporter plasmid together with expression plasmid of TAF$_{II}$105ΔC. Luciferase activity of three independent experiments was measured. *D*, TAF$_{II}$105 mediates B cell octamer transcription *in vitro*. *Upper panel*, schematic representation of reporter plasmids used for *in vitro* transcription assay. IgH TATA is a minimal IgH core promoter element [(−47)-(+1)], 2xOct/IgH TATA contains two tandem octamer motifs upstream to the IgH promoter, and MLP TATA contains the adenovirus major late core promoter that is used as specificity control. *Lower panel*, the activity of the promoters was examined *in vitro* using Daudi B cell crude nuclear extract in the absence or presence of TAF$_{II}$105 and HBV-core-specific antibodies as indicated at the *top* of the *lanes*. The reaction of *lane 1* was carried out with IgH TATA reporter, whereas in *lanes 2–4*, 2xOct/IgH TATA was used. The MLP TATA reporter was used in *lanes 5–7*.

3–4 days. Pools of G418-resistant cells were analyzed by Western blotting using the anti-TAF$_{II}$105 polyclonal antibodies.

RESULTS

TAF$_{II}$105 Is Involved in Octamer-dependent Transcription in B Cells—As a first step in testing the possible involvement of TAF$_{II}$105 in octamer-dependent transcription, we established a pool of S194 B cells constitutively expressing hTAF$_{II}$105. These and control cells were transfected with two different octamer-dependent luciferase reporter genes (Fig. 1*B*), one contains four tandem octamer sites and the other has a single octamer site upstream to a minimal core promoter element. As shown in Fig. 1*B*, octamer-dependent luciferase activity is higher in TAF$_{II}$105-expressing cells. The activities of reporter plasmids containing mutated octamer motifs or lacking octamer motif were almost undetectable and were unaffected by TAF$_{II}$105 expression (columns 5–8). Although the fold of induction of octamer activity was not high, it was highly reproducible. Considering the results shown in the subsequent figures, it may be explained by the low level of ectopic expression TAF$_{II}$105 achieved in S194 cells relative to human 293 cells (Fig. 1*A*).

Next, we tested the potential of a C-terminal truncated form of TAF$_{II}$105 (TAF$_{II}$105ΔC) to inhibit octamer transcription. This mutant is deleted of the C-terminal TFIID-binding domain and was previously shown to act as a dominant negative mutant of TAF$_{II}$105 function (19). BJAB and S194 B cell lines were transfected with octamer-dependent reporter plasmid together with expression plasmid for TAF$_{II}$105ΔC. As shown in Fig. 1*C*, TAF$_{II}$105ΔC inhibited octamer transcription in both cell lines.

To examine more directly the involvement of TAF$_{II}$105 in octamer-dependent transcription, we performed *in vitro* transcription reactions using a reporter plasmid composed of two octamer elements upstream of a minimal IgH core promoter (Fig. 1*D*, *upper panel*) and nuclear extract prepared from the Daudi B cell line. As expected, the transcription level of the octamer-containing reporter plasmid was significantly higher than the core promoter alone (Fig. 1*D*, *lower panel*, *lanes 1* and *2*) confirming that octamer-binding proteins mediate octamer transcription *in vitro*. Antibodies directed against TAF$_{II}$105 N-terminal domain decreased 95% of octamer-dependent transcription (compare *lanes 2* and *3*), whereas control antibodies have no effect on octamer transcription (compare *lanes 2* and *4*). By contrast, less then 8% reduction was observed on tran-

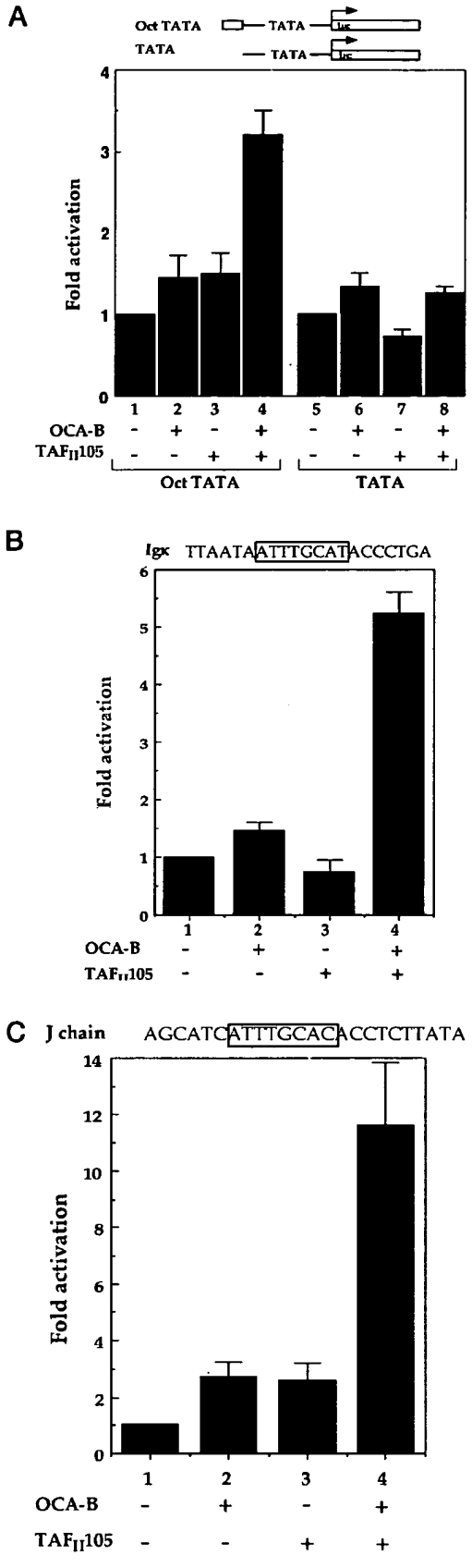

FIG. 2. **Stimulation of octamer-dependent activity by $TAF_{II}105$ and OCA-B.** *A*, reporter plasmids containing a single octamer site (*columns 1–4*) or TATA box only (*columns 5–8*) were transfected into human 293 cells together with either empty expression vector or expression plasmids for OCA-B or $TAF_{II}105$ as indicated at the *bottom* of each *lane*. Transcriptional activation of B cell-specific promoters by OCA-B and $TAF_{II}105$ was analyzed by transfection assays using luciferase reporter plasmids driven by the promoters of Igκ light chain (*B*) and J chain (*C*). Each promoter contains an octamer sequence as shown. These promoters were introduced into 293 cells along with limiting amounts of OCA-B expression plasmid (*columns 2* and *4*) and $TAF_{II}105$ (*columns 3* and *4*), and luciferase activity was measured. The activity of the reporter alone was normalized to 1. The amount of CMV-derived vector in each transfection was kept constant. Expression of all effector plasmids was confirmed by Western blot (data not shown). The results of these transfection experiments are the average of three independent experiments with similar results.

scription mediated by the adenovirus major late core promoter, consistent with our previous finding that approximately 10% of the TFIID contain $TAF_{II}105$ in Daudi nuclear extract (MLP, *lanes 5–7*). Taken together, these results suggest that $TAF_{II}105$ is involved in octamer-mediated transcription in B cells.

$TAF_{II}105$ and OCA-B Cooperate to Activate Octamer Transcription on Artificial and Native B Cell-specific Promoters—In B cells, gene activation through an octamer motif is regulated by two octamer-specific binding proteins Oct1 and Oct2 and a B cell-specific cofactor OCA-B (36). OCA-B is recruited to the octamer site by direct interaction with the POU domains of Oct1 and Oct2 and activates octamer-specific transcription by providing a transcription activation function to Oct1 and Oct2 (32–34). To examine the role of $TAF_{II}105$ in octamer-mediated transcription, the effect of OCA-B and $TAF_{II}105$ expression on octamer-dependent transcription activity was tested using transient transfection experiments in the human kidney fibroblast cell line 293. In these cells OCA-B is brought to the promoter by the ubiquitously expressed Oct1 protein. Luciferase reporter plasmid containing a minimal core promoter element and an upstream octamer site were cotransfected together with OCA-B or $TAF_{II}105$ expression plasmids and luciferase activity was determined. To observe a coactivation effect by both proteins, we used limiting amounts of each factor. As shown in Fig. 2*A*, expression of $TAF_{II}105$ and OCA-B resulted in a marked increase of octamer activity relative to the expression of each of these proteins alone (compare *column 4* with *columns 2* and *3*). This stimulation is specific because coexpression of $TAF_{II}130$, a TFIID subunit related to $TAF_{II}105$, has no stimulatory effect on octamer activity (data not shown). Moreover, $TAF_{II}105$ fails to stimulate the reporter plasmid-lacking octamer site in the presence or absence of OCA-B (*columns 5–8*), ruling out the possibility that $TAF_{II}105$ affects core promoter function.

To examine the role of $TAF_{II}105$ in transcription of physiologically relevant genes, we used a luciferase reporter gene driven by promoters of two B cell-specific genes, immunoglobulin kappa light chain (Igκ) and J chain. The proximal promoter region of both genes contains an octamer site. As expected, the promoter activity of these genes in non-B cells is very low (Fig. 2, *B* and *C*). Cotransfection of either OCA-B or $TAF_{II}105$ with these reporter plasmids, has minor effect on Igκ and J chain promoter activities (Fig. 2, *B* and *C*, *columns 2* and *3*). However, transfection of OCA-B and $TAF_{II}105$ with the B cell-specific reporter genes resulted in strong stimulation of reporter gene activity, suggesting that OCA-B and $TAF_{II}105$ collaborate to stimulate the activity of these promoters. The potentiation of OCA-B activity by $TAF_{II}105$ in the context of the native promoters is greater then that observed with the artificial promoter (Fig. 2*A*), raising the possibility that the organization of the native promoters is more suitable for activation by OCA-B and $TAF_{II}105$. Taken together, these results suggest that $TAF_{II}105$ coactivator function on the octamer element is mediated by OCA-B.

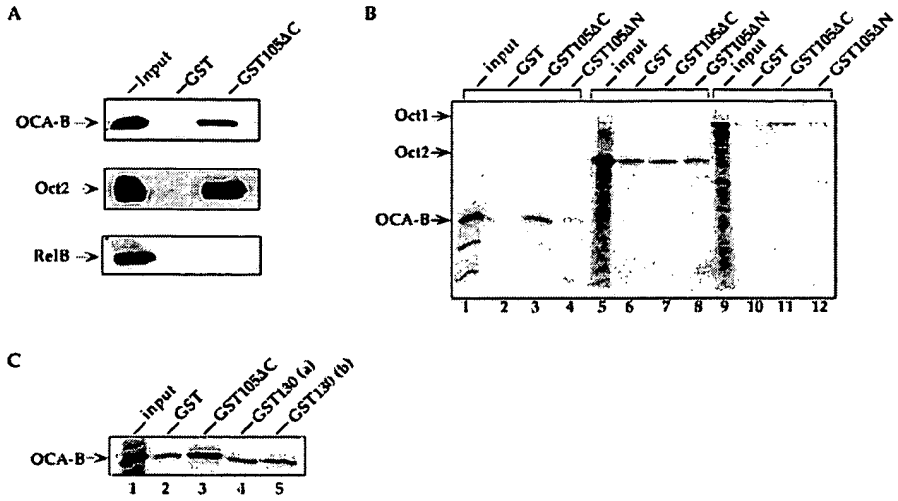

FIG. 3. **Specific interaction of $hTAF_{II}105$ with OCA-B.** *A*, purified $TAF_{II}105$ protein (GST105ΔC, amino acids 1–552) fused to GST and bound to glutathione-Sepharose beads, or control GST-containing beads were used for binding reaction with nuclear extract prepared from the human B cell line BJAB. The bound proteins were eluted by high salt and subjected to SDS-PAGE followed by Western blot analysis using anti-OCA-B, anti-Oct2, or anti-RelB antibodies. Input lanes represent 10% of the total nuclear extract used for the binding. *B*, $TAF_{II}105$ interacts directly with OCA-B through its N-terminal domain. Oct1, Oct2, and OCA-B proteins were translated *in vitro* and labeled with [^{35}S]methionine using rabbit reticulocyte lysate. These proteins were used for binding assay using GST105ΔC described in *A* or GST105ΔN protein (amino acids 551 to end). The bound proteins were eluted, resolved by SDS-PAGE, and autoradiographed. Input lanes represent 10% of the total protein used. *C*, OCA-B interacts specifically with $TAF_{II}105$ but not with $TAF_{II}130$. Translated OCA-B protein was analyzed for the ability to bind the N-terminal domain of $TAF_{II}130$ protein homologous to the N-terminal domain of $TAF_{II}105$. To achieve reasonable levels of expression, $TAF_{II}130$ protein was dissected into two fragments: (a) and (b). Fragment (b) was found to be competent for protein-protein interaction, as it efficiently binds SP1 (data not shown).

OCA-B Interacts with $TAF_{II}105$—To determine the mechanism involved in $TAF_{II}105$ action on octamer transcription, we tested whether octamer factors can interact with $TAF_{II}105$. For this purpose nuclear extract prepared from the B cell line BJAB was incubated with immobilized $TAF_{II}105$ (N terminus, amino acids 1–552), and the bound proteins were analyzed by Western blot using antibodies specific to Oct2 and OCA-B. As shown in Fig. 3*A*, both Oct2 and OCA-B were retained on $TAF_{II}105$-containing beads but not on GST beads. The binding of Oct2 and OCA-B is specific because RelB, another B cell transcription factor, failed to associate with GST-$TAF_{II}105$ on the same experiment.

Because OCA-B is complexed in B cells with Oct1 and Oct2, we tested which of these proteins binds directly to $TAF_{II}105$. Each of the proteins Oct1, Oct2, and OCA-B was *in vitro* translated in rabbit reticulocytes lysate and used for binding assay with Sepharose-bound N-terminal or C-terminal fragments of $TAF_{II}105$. As shown in Fig. 3*B*, among the proteins tested, only OCA-B specifically and efficiently interacts with the N-terminal domain of $TAF_{II}105$. The binding of OCA-B to the $TAF_{II}105$ N terminus is specific because it does not interact with protein fragments derived from the N terminus of $hTAF_{II}130$ (Fig. 3*C*), which is consistent with the low level of homology between $TAF_{II}105$ and $TAF_{II}130$ within this region. Considering the inhibition of octamer activity in B cells by a C-terminal truncated form of $TAF_{II}105$ (Fig. 1*C*) and the direct association of OCA-B with this domain, it is reasonable to assume that this mutant exerts its inhibitory effect by competing with native $TAF_{II}105$ protein for OCA-B binding.

$TAF_{II}105$-OCA-B Interaction Is Important for Octamer Transcription—To obtain further evidence that the effect of $TAF_{II}105$ on octamer-dependent transcription is mediated by OCA-B, we have constructed a mutant of OCA-B lacking the last 26 amino acids (OCA-B 1–230) and analyzed it for $TAF_{II}105$ binding capacity and transcription activity. As shown in Fig. 4*A*, deletion of the last 26 residues significantly reduced $TAF_{II}105$ binding. This mutant is incapable of stimulating octamer transcription in 293 cells (Fig. 4*B*). Consistent with $TAF_{II}105$ reduced binding capacity, co-expression of $TAF_{II}105$ with the OCA-B 1–230 mutant fails to enhance its activity (Fig. 4*B*). These results suggest that the interaction between $TAF_{II}105$ and OCA-B is important for activation of octamer-dependent transcription.

Previous studies have indicated that OCA-B contains two transcription activation domains. One domain is mapped to position 65–122, and the second domain resides within the extreme C-terminal region (37–39). To determine which of the activation domains is involved in $TAF_{II}105$ coactivation function and to further confirm that OCA-B directly mediates $TAF_{II}105$ effect, different OCA-B domains were fused to the yeast Gal4 DNA binding domain (Fig. 5*A*). These Gal4 fusion proteins were cotransfected with luciferase reporter gene driven by Gal4 sites upstream to a minimal core promoter. Gal4 DNA binding domain alone (Gal4-DBD) lacks transcription activity and is not induced by $TAF_{II}105$ (Fig. 5*B*, *columns 3* and *4*). OCA-B mutants 1–230 and 1–122 but not 1–100, stimulate the reporter activity (*columns 5*, *7*, and *9*) suggesting that residues within the region of 100–122 of OCA-B are crucial for the N-terminal activation function. However, these C-terminal truncated proteins are not responsive to $TAF_{II}105$ expression (*columns 6*, *8*, and *10*). An N-terminal truncation of OCA-B (amino acids 100–256) also contains a strong activation domain (*column 11*). The activity of this mutant is elevated by $TAF_{II}105$ expression (*column 12*), confirming that coactivation by $TAF_{II}105$ is mediated by the C-terminal activation domain. Interestingly, OCA-B mutant 1–230 is active in the context of fusion with Gal4 DBD, whereas it is inactive in the context of the native protein, raising the possibility that the N-terminal activation domain of OCA-B 1–230 is hidden and becomes exposed in the fusion form.

DISCUSSION

Detailed analysis of B cell-specific transcription regulation by the octamer motif revealed the involvement of two octamer-binding factors, the ubiquitous Oct1 and the B cell-restricted Oct2. These factors are not sufficient to reconstitute a high level of octamer activity observed in B cells. This activity is fulfilled by OCA-B, a B cell-specific cofactor (also termed OBF-1

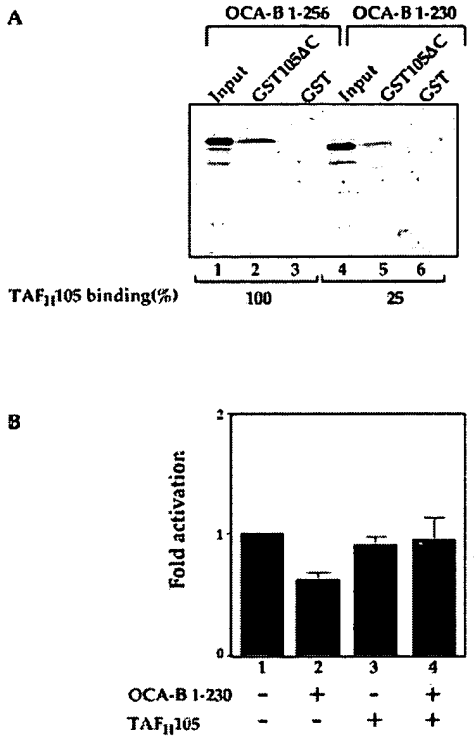

FIG. 4. **Deletion of the last 26 amino acid residues of OCA-B severely reduced $TAF_{II}105$ binding and its transcription activity.** *A*, binding reaction between immobilized $TAF_{II}105$ (GST105ΔC) or GST proteins and *in vitro* translated and labeled wild type OCA-B 1–256 (*lanes 1–3*) or mutant OCA-B 1–230 (*lanes 4–6*). The bound proteins were washed, eluted, and subjected to SDS-PAGE followed by autoradiography. Input lanes represent 10% of the total protein used for the binding. The binding of wild type OCA-B to $TAF_{II}105$ relative to the input represent 100% of binding. *B*, effect of the truncated form of OCA-B (1–230) on octamer transcription. 293 cells were cotransfected with the single octamer-reporter construct and expression plasmids for OCA-B 1–230 (*columns 2* and *4*) and $TAF_{II}105$ (*columns 3* and *4*). Expression of mutated OCA-B was verified by Western blot using anti-HA antibodies (data not shown). Luciferase activity of the reporter cotransfected with empty expression vector (*column 1*) was normalized to 1.

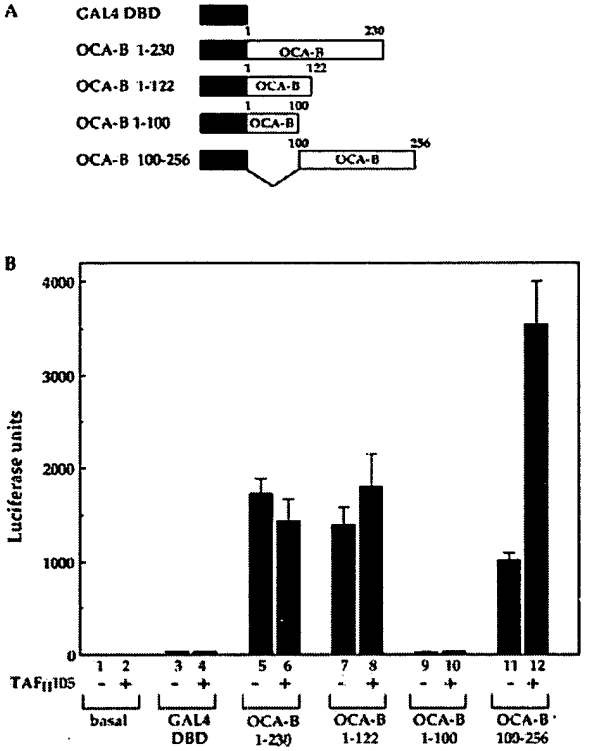

FIG. 5. **The C-terminal activation domain of OCA-B mediates $TAF_{II}105$ effect and directs $TAF_{II}105$ binding.** *A*, schematic presentation of GAL4 fusion protein series with different OCA-B domains. *B*, transcription activity of the Gal4 chimeric proteins. 293 cells were transfected with a luciferase reporter plasmid containing five tandem Gal4 binding sites together with the indicated Gal4 fusion proteins in the presence (*even-numbered columns*) or absence (*odd-numbered columns*) of $TAF_{II}105$ expression plasmid. The amount of CMV-derived vector in each transfection assay was kept constant. The expression of the different OCA-B mutants and $TAF_{II}105$ was verified by Western blot (data not shown).

and Bob1) that is specifically brought to octamer sites by the Oct1 and Oct2 proteins (32–34). In the present study we provide evidence that octamer-dependent activity in B cells is more complex and also involves the B cell-enriched TFIID subunit $TAF_{II}105$ as transcription coactivator for octamer-binding complexes.

Using *in vitro* transcription studies we show that octamer-mediated transcription, reconstituted with B cell nuclear extract, is $TAF_{II}105$-dependent. Protein-protein interaction assays and reporter gene analysis revealed that OCA-B but not Oct1 or Oct2 directly binds $TAF_{II}105$ and that this interaction is essential for stimulation of octamer activity. Furthermore, we show that cooperation between OCA-B and $TAF_{II}105$ is more efficient on native B cell-specific promoters carrying the octamer motif. Consistent with a coactivator model, this interaction is mediated by the main OCA-B activation domain and the N-terminal coactivation domain of $TAF_{II}105$. Given the high concentration of OCA-B and $TAF_{II}105$-TFIID in the nucleus of B lymphocytes, these two proteins are likely to efficiently associate and stimulate the transcription of specific octamer-containing target genes in B cells. Because OCA-B stimulates transcription in B cells only through proximal octamer sites (38), it will be interesting to determine whether $TAF_{II}105$ is also involved in transcription activation of a distally located octamer motif that requires Oct2 and another unidentified B cell-restricted cofactor (40).

Unlike core TFIID-associated factors, $TAF_{II}105$ is found in a relatively small portion of TFIID complexes, 5–10% in certain mature B cell lines and 0.5–1% in non-B cells (30). Therefore, $TAF_{II}105$-containing TFIID is likely to be involved in transcription regulation of small fraction of genes. Given that the amount of TFIID in the cell is limiting, activators capable of $TAF_{II}105$ binding might have an advantage in competing for limiting TFIID and can more efficiently recruit $TAF_{II}105$-TFIID to their gene target. This is particularly important for genes that should be rapidly induced and highly transcribed such as immunoglobulin genes following antigenic stimulation, or as we have previously shown, tumor necrosis factor-α-induced anti-apoptotic genes (19).

The last C-terminal 26 amino acid residues of OCA-B contain an activation domain crucial for OCA-B function. Deletion of this region abrogates the ability of OCA-B to stimulate octamer-dependent transcription. This result is similar to an earlier report in which mutation of acidic amino acid residues within this region significantly impaired OCA-B activity *in vitro* and in transfected HeLa cells (39). Consistent with the notion that direct interaction between $TAF_{II}105$ and OCA-B is involved in octamer transcription *in vivo*, $TAF_{II}105$ failed to stimulate transcription activation by the OCA-B protein lacking the C-terminal domain (OCA-B 1–230) and to efficiently bind this mutant protein.

Besides the C-terminal activation domain, another activation domain within OCA-B was identified and mapped to amino

acid residues 65–122 (37, 38). Our studies revealed that the N-terminal activation domain is not targeted by $TAF_{II}105$. On the basis of the failure of OCA-B 1–230 to stimulate transcription and the facts that larger C-terminal deletions of OCA-B (37) as well as C-terminal truncated Gal4 fusion protein of OCA-B (1–230 and 1–122) are transcriptionally active, it is possible that the region within amino acids 192–230 contains an activation inhibitory function.

Previous biochemical analysis of OCA-B activity revealed that the USA component PC4 is required for transcription activation by OCA-B in a partially purified transcription system (39). In addition, using a PC4-depleted HeLa nuclear extract, these authors also revealed the existence of an additional and redundant OCA-B coactivator activity. It should be noted that cooperation of $TAF_{II}105$ and OCA-B for transcriptional activation in HeLa cells was not observed (data not shown). These findings together with the present study indicate that OCA-B can utilize alternative pathways for transcription activation in different cell types. It is also possible that *in vivo*, under some circumstances, different OCA-B coactivators might cooperate during different steps of the transcription activation process. Consistent with this notion is the finding that the coactivation function of PC4 is dependent on TAFs (41, 42).

Certain *in vitro* transcription studies indicated that TAFs are absolutely required for transcription activation (9, 10). However, recent studies revealed a mechanism of transcription activation that is TAF-independent (43, 44). The existence of transcription activation pathways with redundant coactivator activities raises the interesting possibility that *in vivo*, gene-specific regulation is determined by the combinatorial action of different sets of coactivators, therefore providing an additional level of control.

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